Detoxication of Vinca Alkaloids by Human P450 CYP3A4-Mediated Metabolism: Implications for the Development of Drug Resistance¹

DENGGAO YAO, SHAOHONG DING, BRIAN BURCHELL, C. ROLAND WOLF, and THOMAS FRIEDBERG
Imperial Cancer Research Fund, Molecular Pharmacology Unit, and Biomedical Research Centre (D.Y., S.D., C.R.W., T.F.); and Department of Cellular and Molecular Pathology (B.B.), Ninewells Hospital and Medical School, Dundee, United Kingdom
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ABSTRACT
Vinca alkaloids are important chemotherapeutic agents, and their pharmacokinetic properties display significant interindividual variations, possibly due to CYP3A4-mediated metabolism. We have evaluated the relevance of this metabolism for the chemotherapeutic and the toxicological properties of these drugs. Analysis was performed using Chinese hamster ovary cell lines that expressed either CYP2D6 or CYP3A4. The latter cells metabolized vinblastine with a turnover number of 0.4 min⁻¹, resulting in a decreased cytotoxicity of this compound. Whereas vincristine and vinblastine at a concentration of 100 nM killed more than 90% of the parental cells, more than 50 and 35%, respectively, of cells that coexpressed CYP3A4 and cytochrome P450 (P450) reductase survived these treatments. No additional increase in cytotoxicity was noted above 100 nM. Similarly, preincubation of vinblastine with bacterial membranes that contained recombinant CYP3A4 and P450 reductase decreased the cytotoxicity of vinblastine for parental Chinese hamster ovary cells. We also demonstrate that the presence of vinblastine in a coculture of cells that expressed β-galactosidase together with cells that expressed CYP3A4 strongly selected for the latter cells, resulting in an increased level of CYP3A4 in the surviving cell population. Similarly, treatment of the human colon adenocarcinoma cell line LS174T with vinblastine selected for a cell population with higher levels of endogenous CYP3A4 as revealed by immunohistochemistry without simultaneous increase of multidrug resistance protein 1 (MDR1). This is the first evidence that tumor P450s have the potential to contribute to the development of drug resistance during chemotherapy.

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ABBREVIATIONS: P450, cytochrome P450; CHO, Chinese hamster ovary; hOR, NADPH-P450 reductase; MDR1, multidrug resistance protein 1; DMEM, Dulbecco’s modified Eagle’s medium; DMSO, dimethyl sulfoxide.
P450-mediated metabolism of these compounds showed a broad interindividual variability that correlated with the CYP3A levels in human liver microsomes. This may explain the large interindividual variation in the clinical pharmacokinetics of vinca alkaloids. However, in the absence of data on the biological activity of the resulting primary or secondary metabolites, the relevance of this observation for the desired and adverse effects of vinca alkaloids remains unfortunately unknown.

Tumor cells may become refractory to treatment with vinca alkaloids by a variety of mechanisms. These include alterations in the structure of tubulin proteins (Houghton et al., 1985) or increased expression of the multidrug resistance protein MDR1 (P-glycoprotein), which is known to mediate the cellular export of several drugs (Ueda et al., 1987). In vitro, prolonged treatment of cells with compounds that are substrates for MDR1 often selects for cells that overexpress this protein. It is intriguing that most substrates of the major hepatic P450 isoform CYP3A4 are either transported by MDR1 or are modulating the activity of this transporter. For example, vinca alkaloids, colchicine, and cyclopodine are substrates for both CYP3A4 and MDR1, whereas nifedipine and verapamil are substrates for the P450 but inhibitors for MDR1 (Kivisto et al., 1995; Schuetz et al., 1996). It could be speculated that both proteins coevolved to protect cells from toxic noxes from environmental toxins.

Some P450s, including CYP3A4, have been shown to be overexpressed in tumors (Murray et al., 1993a,b). However, mechanisms leading to the development of drug resistance by the overexpression of proteins have been mainly studied in cell culture. Because it is known that the expression of several P450 isoforms is usually strongly decreased on cultivation of cells (LeCluyse et al., 1986; Maurel, 1996), P450-mediated drug resistance may have escaped detection. In this study, we have determined the role of P450-mediated metabolism in the toxicity and in the development of drug resistance to vinca alkaloids.

### Materials and Methods

**Cell Culture and Establishment of Cell Lines.** Cell lines described in this study were derived from a dihydrofolate reductase negative (DHFR-) Chinese hamster ovary (CHO) cell line, designated DUKXB11 (Kaufman, 1990). The isolation of the cell lines that stably expressed CYP3A4 either alone or together with hOR has been described recently (Ding et al., 1997). A similar procedure was used to establish the CHO cell lines that expressed the cell line that coexpressed CYP2D6 and hOR. For mass propagation, the cells that coexpressed P450s and hOR were maintained in the presence of rifampicin for 3 days, after which the medium was exchanged for fresh medium containing 10% FBS and grown for another 3 days. The cells were harvested and total cell protein was prepared by sonication and analyzed by immunoblotting using anti-CYP3A4 antibody or the anti-MDR1 JSB-1 (Chemicon, London, UK) antibody and horseradish peroxidase-coupled secondary antibody (SAPU, Carluke, Scotland). The blot was developed using an enhanced chemiluminescence kit (ECL; Amersham, Buckinghamshire, UK).

A similar experiment was performed on the human colon adenocarcinoma cell line LS174T (catalog no. 87060401; European Collection of Animal Cell Culture, Salisbury, UK), which has been derived from the LS180 cell line. The latter cell line has been previously shown to express CYP3A4 (Schuetz et al., 1996). LS174T cells were seeded and grown for 24 h in RPMI medium containing 10% fetal calf serum to a confluence of approximately 20%. Subsequently cells were grown in fresh medium containing 10 μM rifampicin for 3 days, after which the medium was exchanged for medium containing 10 μM rifampicin and different concentrations of vinblastine. After additional incubation for 3 days, total cellular protein was isolated and analyzed by immunoblotting as described above.

**Determination of Selective Survival in Recombinant Cells.** To determine whether CYP3A4 expression confers selective growth advantage in the presence of vinblastine, the following experiments were performed with recombinant CHO cells. The CHO/GAL cell line and the cell line that expressed CYP3A4 and hOR were cultured either separately or in coculture in the absence or presence of vinblastine (900 nM) for 24 h. β-Galactosidase was visualized by incubating the cells with X-gal according to the method provided by the supplier of the vector pSV-β-gal (Promega, Southampton, UK). After this, the cells were permeabilized with 1% Triton X-100, fixed with 3% paraformaldehyde in PBS, subsequently reacted with anti-CYP3A4 antibody (raised in rabbit), washed, and incubated with
horseradish peroxidase-coupled anti-rabbit IgG. The slides were developed with the Dab fast kit (Sigma), containing diaminobenzidine.

**Determination of CYP3A4-Mediated Resistance in Tumor Cells.** To investigate whether CYP3A4 expression in human tumor cells contributes to drug resistance to vinblastine, the following experiment was performed: LS174 cells were seeded and grown for 24 h in RPMI medium containing 10% fetal calf serum to a confluence of 20%. Subsequently, either rifampicin (final concentration 10 μM) or solvent [dimethyl sulfoxide (DMSO), final concentration less than 0.1%] was added. After 3 days, vinblastine (final concentration 80 nM) or DMSO (final concentration less than 0.2%) was added. After another 3 days, cells were fixed and permeabilized with formaldehyde and Triton X-100 as described above. Cells were reacted with anti-CYP3A4 antibody. For immunofluorescence, cells were incubated with fluorescein isothiocyanate-labeled secondary antibody. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (Zhang et al., 1999).

**Preincubation of Vinblastine with Recombinant P450s.** Vinblastine was preincubated either with CHO cells coexpressing CYP3A4 and hOR or with membranes isolated from E. coli that coexpressed recombinant P450 isoforms and hOR. Subsequently, the cytotoxicity of the preincubation mix was assayed using parental CHO cells. For preincubation with mammalian cells, 1 × 106 cells were seeded and grown for 24 h. Fresh medium containing 1 μM vinblastine was added and incubated for 3 days. This preincubation mixture was used for the cytotoxicity assay as indicated above after dilution with medium (DMEM) to achieve the final concentrations given in Fig. 3. For preincubation with membranes isolated from E. coli, vinblastine (30 μM) was incubated in 0.1 M phosphate buffer, pH 7.4, containing 30 mM Mg2+ in the presence and absence of 0.5 mM NADPH. Each incubation contained 500 pmol of recombinant P450. Preincubation was carried out at 37°C for 4 h. Subsequently, this preincubation mixture was sterile filtered through 0.2-μm filters. Cytotoxicity was assayed as described above using parental CHO cells after dilution of the mixture with medium to the concentrations indicated in Fig. 4.

**Results**

**P450-Mediated Metabolism of Vinca Alkaloïds.** In this investigation, we used CHO cell lines that coexpressed hOR together with CYP2D6 or CYP3A4. CHO cells, which coexpressed CYP3A4 together with P450 reductase (hOR), have been described recently (Ding et al., 1997). In addition, we used cells that expressed CYP3A4 but no hOR. The levels of spectrally detected P450 and associated enzyme activities toward prototypical substrates for these cell lines are given in Table 1. The parental cell line did not contain spectrally detected P450 or display P450 enzyme activity toward the substrates given in Table 1. In the recombinant cell lines, the expression levels of the P450 isoforms and their activities were either similar or higher than those found in human liver microsomes. The exception was the cell line that expressed CYP3A4 alone, which had a 10-fold lower testosterone 6β-hydroxylase activity than cells that contained CYP3A4 and hOR.

The metabolism of [3H]vinblastine was determined using total cellular protein isolated from the recombinant cell lines. In the presence of NADPH, cellular protein isolated from cells that coexpressed CYP3A4 and hOR formed a metabolite that eluted later than vinblastine. From this experiment, the P450 enzyme activity toward vinblastine was calculated to be 8.5 pmol/min/mg of protein, which corresponds to a turnover number of 0.4 min⁻¹. These values are similar to the microsomal hepatic P450 enzyme activity toward vinblastine (Zhou et al., 1993a). This metabolite was not detected in the parental CHO cells or in cells that expressed hOR or CYP3A4 alone. It was also not detectable in cells that coexpressed CYP2D6 and hOR. The identity of this metabolite is not known (Zhou et al., 1993a).

**Role of P450s in the Toxicity of Vinca Alkaloids.** The cytotoxicity of vincristine and vinblastine was determined by incubating the recombinant cell lines with increasing concentrations of the vinca alkaloids for 3 days. Cytotoxicity curves for vincristine were rather similar for all cell lines tested up to a concentration of 3.6 nM (Fig. 1). At this concentration up to 80% of the cells survived. The IC₅₀ for all cell lines was approximately 11 nM. However, beyond this concentration, which is much lower than the systemic concentration (50–100 nM) reached during a bolus injection (Rowinsky and Donehower, 1996), an additional increase in the concentration of vincristine did not increase its cytotoxicity for the cell line that coexpressed CYP3A4 and hOR. In contrast, more than 90% of the cells that expressed CYP2D6 together with hOR or the hOR alone were sacrificed at a concentration of 300 nM. Note that coexpression of CYP3A4 and hOR did not shift the entire cytotoxicity curve to the right.

The expression of CYP3A4 and hOR also clearly reduced the cytotoxicity of vinblastine at higher doses (higher than 11 nM); however, the effect appeared to be less pronounced than with vincristine (Fig. 2). Again, coexpression of CYP3A4 and hOR did not shift the entire cytotoxicity curve to the right (see Discussion).

To further verify the involvement of CYP3A4 in the detoxification of vinca alkaloids, we studied the effects of CYP3A4 inhibitors on the cytotoxicity of vinblastine. In these experiments, the inhibitors were used at a concentration that inhibited the testosterone 6β-hydroxylation activity of CYP3A4 in intact CHO cells by at least 70%. Surprisingly, we found that ketoconazole (1 μM) and troleandomycin (10 μM) strongly increased the cytotoxicity of vinblastine already for the parental cells (data not shown). Therefore, we did not evaluate the effects of CYP3A4 inhibitors on cells that contained this P450 but used an alternative approach to address this issue. For this, we preincubated vinblastine with membranes containing recombinant P450 isoforms and P450 reductase, and assayed the effects of this preincubation on the cytotoxicity in parental CHO cells. Membranes were derived from CHO cells that expressed CYP3A4 and hOR (Fig. 3) or from E. coli that coexpressed hOR together with either

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**Table 1**

Characterization of the recombinant cell lines used in this work

<table>
<thead>
<tr>
<th>Recombinant Protein Expresses</th>
<th>P450 Level</th>
<th>Catalytic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/mg of total cellular protein</td>
<td>pmol/mg/min total cellular protein</td>
</tr>
<tr>
<td>CYP2D6 and hOR</td>
<td>22</td>
<td>905</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>27</td>
<td>77</td>
</tr>
<tr>
<td>CYP3A4 and hOR</td>
<td>21</td>
<td>900</td>
</tr>
</tbody>
</table>
CYP1A2 or CYP2C8 or CYP2D6 or CYP3A4 (Fig. 4). Typically, membranes isolated from recombinant E. coli displayed an ethoxyresorufin O-deethylase activity of 60 pmol/min/mg of protein (CYP1A2 membranes), a taxol 6α-hydroxylase activity of 130 pmol/min/mg of protein (CYP2C8 membranes), a bufuralol 1'-hydroxylase activity of 1,200 pmol/min/mg of protein (CYP2D6 membranes), and a testosterone 6β-hydroxylase activity of 12,000 pmol/min/mg (CYP3A4 membranes). It was found that preincubation of vinblastine with mammalian cells containing CYP3A4 significantly shifted the cytotoxicity curve to the right compared with preincubation with parental cells (Fig. 3). A similar effect was observed with bacterial membrane fractions containing CYP3A4 and hOR in the presence but not in the absence of NADPH (Fig. 4A). This was not observed for membranes containing the other three P450 isoforms (Fig. 4, B–D). In these experiments, each preincubation contained 500 pmol of the recombinant P450.

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P450s and Development of Drug Resistance. The role of CYP3A4 for the development of drug resistance in a heterogeneous cell population during treatment with chemotherapeutics was evaluated in a coculture of a cell line that coexpressed CYP3A4 and hOR together with a cell line that expressed β-galactosidase. The selective cytotoxicity of vinblastine was determined either histochemically by staining for CYP3A4 and β-galactosidase (see Materials and Methods). Histochemical analysis demonstrated that cells that reacted with the CYP3A4 antibody, as revealed by brown staining, survived treatment with vinblastine much better than the cells that expressed β-galactosidase as revealed by blue staining (Fig. 5).

Support for the role of CYP3A4 in drug resistance is also given by the observation that vinblastine treatment of cells that expressed recombinant CYP3A4 alone or together with recombinant hOR led to an increase of immunodetectable CYP3A4 in total cellular protein isolated from these incubations (Fig. 6A, cf. lanes 4 versus 5 and lanes 6 versus 7), most likely, by eliminating cells that contained low levels of CYP3A4. It is important to note that mdr1 was not detectable in CHO cells, not even after treatment with vinblastine (Fig. 6C).
Fig. 3. Cytotoxicity of vinblastine after preincubation with mammalian cells. Vinblastine (1 μM) was preincubated with parental CHO (DUKXB11) cells (○) or with cells that coexpressed CYP3A4 and hOR (▲) for 3 days. Subsequently, the preincubation mix (as defined under Materials and Methods) was diluted to the concentrations indicated in the figure and used in a cytotoxicity assay as described in the legend to Fig. 2, using parental CHO cells as the target. The S.D. was less than 10% of the means. **, significantly different (P ≤ .01) from control (DUKXB11 cells).

Fig. 4. Cytotoxicity of vinblastine after preincubation with E. coli membranes containing recombinant P450s. Vinblastine (30 μM) was preincubated in the absence (open symbols) or presence (closed symbols) of NADPH with membranes isolated from E. coli expressing the hOR together with the following P450 isoforms: CYP3A4 (A); CYP1A2 (B); CYP2C8 (C); and CYP2D6 (D). After incubation for 4 h at 37°C, the mixture (composition see Materials and Methods) was sterile filtered, diluted to the concentration indicated in the figure, and subsequently tested for cytotoxicity using parental CHO cells. The S.D. was less than 10% of the means. *, significantly different (P ≤ .05) from control (DUKXB11 cells).

To evaluate whether CYP3A4 also mediates the development of drug resistance in human cancer cells, the colon adenocarcinoma cell line LS174T was exposed to vinblastine. Subsequently, the levels of CYP3A4 were determined by immunoblotting (Fig. 6B). No immunodetectable CYP3A4 was seen in untreated and vinblastine-treated LS174T cells. CYP3A4 was only detectable after exposure to the well-known CYP3A4 inducing agent rifampicin. Additional treatment of these cells with increasing concentrations of vinblastine led to a strong rise in the level of endogenous CYP3A4 in the surviving cell population. MDR1 was not detectable in 20 μg of total cellular protein isolated from untreated and treated LS174T cells (Fig. 6C). Under these conditions, 5 μg of total cellular protein isolated from a MDR1 overexpressing human ovarian cancer cell line yielded a strong signal.

Immunohistochemical detection of CYP3A4 in untreated LS174T cells showed some vesicular staining near the nuclear envelope (Fig. 7). Surprisingly, treatment with rifampicin resulted in a strong induction of CYP3A4 only in some cells. On additional treatment with vinblastine, surviving cells, which mostly grew as clones, displayed a high level of CYP3A4.

**Discussion**

Using human liver microsomes, previous studies have demonstrated that vinca alkaloids were metabolized by CYP3A proteins, and no evidence for the involvement of other P450 isoforms was found (Zhou et al., 1993a,b). We confirmed these results by showing that cells that coexpressed CYP3A4 and hOR were able to metabolize vinblastine. In our experiments, metabolism of vinblastine could not be detected in membranes isolated from E. coli that expressed either CYP1A2 or CYP2C8 or CYP2D6 (data not shown). These results strongly suggest that the interindividual variability in the pharmacokinetics of vinblastine are governed by the variation in CYP3A4 levels as proposed previously. Until now, however, the role of P450-catalyzed metabolism for the systemic toxicity of vinca alkaloids and the toxic potential of the resulting metabolites was not known.

Our data demonstrate, using several experimental approaches, that CYP3A4 detoxifies vinca alkaloids. One may argue that the increased resistance of the CYP3A4-expressing cell lines to vinca alkaloids may be unrelated to P450 expression but could have been due to increased expression of mdr1 in the recombinant cell lines. Expression of mdr1 was not detectable in the various recombinant cell lines used in this investigation (Fig. 6C), confirming that CHO cells contain only low levels of mdr1 (Turner and Curtin, 1996; Petriz et al., 1997), which are not increased to a detectable level during establishment of the cell lines used in this study. MDR1 expression also remained undetectable after treatment of the cell lines with vinblastine. However, it should be mentioned that attempts to study the effects of inhibitors of CYP3A4 (troleandomycin and ketoconazole) on the cytotoxicity of vinblastine were not conclusive, because these compounds already increased the cytotoxicity in the parental cell line, possibly due to an inhibition of drug transport proteins for vinca alkaloids. These transporters may include mdr1 (inhibitable by ketoconazole but not detectable in our cell lines) or alternatively mrp1, which has been shown to transport vinblastine and which is also inhibited by ketoconazole or its derivatives (Siegsmund et al., 1994; Hollo et al., 1996).

These results are in agreement with earlier observations that drug efflux can be reduced in CHO cells by inhibitors of mdr1.
(or related proteins), despite the extremely low level of this protein in this cell line (Turner and Curtin, 1996; Petriz et al., 1997). Because CYP3A4 inhibitors could not be used to confirm the role of CYP3A4 in the detoxication of vinca alkaloids, an alternative approach was used (Figs. 3 and 4). The results from these experiments clearly show that preincubation of vinblastine with membranes containing recombinant CYP3A4 reduced the cytotoxicity of the preincubation mixture compared with preincubations with membranes that were devoid of this P450 isoform.

Surprisingly, in experiments using intact cells, expression of CYP3A4 and hOR did not shift the entire cytotoxicity curves for vinca alkaloids to the right (Figs. 1 and 2). Importantly, however, this shift was observed in experiments that assayed the cytotoxicity of vinblastine after preincubation with CHO cells or with E. coli membranes containing CYP3A4 (Figs. 3 and 4A). It is likely that the initial drop in the cytotoxicity curves (Figs. 1 and 2) for cells that have been transfected with CYP3A4 and hOR was caused by a fraction of cells that inadvertently expressed little CYP3A4. The remaining high expressing cells were then resistant to concentrations of vinca alkaloids up to 1 μM. This increased resistance on expression of CYP3A4 can be explained if the import of vinca alkaloids into the cells was becoming saturated compared with their metabolism by CYP3A4, resulting altogether in a decreased intracellular concentration of the cytotoxic parental compounds. The mean uptake rate for vinblastine into hepatocytes has been reported to be 0.568 pmol/min/10⁶ cells (Zhou et al., 1994), which is more than one order of magnitude less than the CYP3A4-mediated metabolism of this compound in the cell line that expressed CYP3A4 together with hOR. The heterogeneity of CYP3A4 expression levels in the cell line that had been transfected with CYP3A4 and hOR was also revealed by immunostaining (Fig. 5). We were not able to eliminate this heterogeneity despite repeated subcloning. Interestingly, exposure of cells

**Fig. 5.** Effects of vinblastine on a coculture of cells that expresses either CYP3A4 or β-galactosidase. Cells that expressed β-galactosidase (Gal) or CYP3A4 together with hOR (3A4) and a coculture of these two cell lines (Gal/3A4; in a ratio of 2:1) were exposed to vinblastine (+V) at a concentration of 900 nM for 3 days. Subsequently, the cells were stained for β-galactosidase (blue) and for CYP3A4 (brown) as described under Materials and Methods. Also shown is the analysis of the cocultured cell lines that had been grown in the absence of vinblastine (−V).
Influence of vinblastine on the level of immunodetectable CYP3A4 in recombinant CHO cells and in intestinal cancer cells. A, CHO cells that expressed recombinant CYP3A4 alone or CYP3A4 and hOR were grown with (+) and without (−) vinblastine (V, 100 nM) for 3 days and, subsequently, the level of CYP3A4 was determined by immunoblotting as described under Materials and Methods. Human liver microsomes (HLM) were included for comparison. In each lane, 20 µg of protein was analyzed. B, human intestinal cancer cell line LS174T was grown in the presence (+) or absence (−) of rifampicin (R) as described under Materials and Methods. Subsequently, the medium was exchanged against medium containing, in addition, vinblastine (V) at the indicated concentrations. After 3 days the levels of CYP3A4 were determined by immunoblotting. Forty micrograms of total cellular lysate from LS174T cells was analyzed. For comparison, cell lysate from CYP3A4 and hOR CHO cells (2.5 µg) was included. C, expression of mdr1 in CHO, CHO (CYP3A4 and hOR), and LS174T cells. Total cellular protein (20 µg) isolated from either CHO or LS174T cells was analyzed by immunoblotting using anti-MDR1 (JSB-1) primary antibody, which, according to the supplier (Chemicon, London, UK), will recognize Chinese hamster mdr1 and human MDR1. As positive control, total cellular lysate (5 µg) isolated from the MDR1-overexpressing and vinblastine-resistant human ovarian cell line A1847 (Louie et al., 1986) was used. CHO (CYP3A4 and hOR) cells were also analyzed after exposure to vinblastine (100 nM) for 3 days. LS174T cells were either untreated or treated with rifampicin (10 µM) or vinblastine (80 nM) or rifampicin (3 days exposure) followed by vinblastine (subsequent exposure for 3 days).

that expressed CYP3A4 to vinblastine led to an elevated level of CYP3A4 as revealed by immunoblotting (Fig. 6A) most likely by eliminating cells that expressed low levels of this P450.

The latter result also suggests that CYP3A4-mediated detoxication of vinca alkaloids is not only relevant to the systemic toxicity of these anticancer drugs, but also implied that CYP3A4 could be involved in the development of drug resistance of tumors during chemotherapy. This was also confirmed by treating a coculture of cells that expressed β-galactosidase together with cells that expressed CYP3A4 with vinblastine. In this experiment, the latter cell line survived preferentially. This experiment also establishes that depletion of vinblastine in the medium by CYP3A4-mediated metabolism is not responsible for the decreased cytotoxicity of vinca alkaloids. It is more likely that this metabolism reduces the intracellular concentration of these compounds, which have been shown to diffuse slowly through cellular membranes (see above; Zhou et al., 1994).

While the above experiments relied on recombinant cell lines, the data on the effect of vinblastine on the CYP3A level in LS174 tumor cells (Figs. 6B and 7) strongly indicate that CYP3A plays a role in drug resistance in human tumor cells. Reverse transcriptase-polymerase chain reaction using primers that will only amplify CYP3A4 but not CYP3A5 and CYP3A7 verified that CYP3A4 was expressed in LS174 tumor cells (data not shown) even though it cannot be excluded that these cells also express CYP3A7 or CYP3A5. However, the former P450 was not detectable in the related intestinal tumor cell line LS180 and the latter P450 was not inducible by rifampicin in LS180 cells (Schuetz et al., 1996). In our experiments, it was observed that treatment of LS174T cells with rifampicin led to a strong induction of a CYP3A protein, which most likely was CYP3A4 in only some cells with the remaining population showing no effect. However, after treatment with vinblastine for another 3 days, expression was seen in most surviving cells, which often formed clones. Our interpretation of this experiment is that preferentially cells that expressed CYP3A4 survived treatment. An alter-
native interpretation is that vinblastine as a ligand of CYP3A4 stabilized this protein and acted synergistically together with vinblastine to result in a superinduction of P450 in each cell. Even though we cannot exclude this mechanism, one would expect that stabilization of CYP3A4 can only occur in the few cells in which this P450 was previously induced by rifampicin, thus maintaining a heterogeneous expression pattern. This was not observed. Furthermore, it is unlikely that LS174T cells, which displayed high levels of CYP3A4 after treatment with rifampicin, were resistant against vinblastine due to simultaneous induction of MDR1, as this protein was undetectable in untreated cells, and even after treatment with rifampicin or vinblastine (Fig. 6C). In this respect LS174T cells, at least under our culture conditions, appear to behave differently from LS180 cells, which have been shown to respond to treatment with rifampicin with a strong induction of MDR1 (Schuetz et al., 1996).

The relevance of our in vitro study for the in vivo situation remains to be established. It is important to note that P450s of the CYP3A family have been shown to be overexpressed in a variety of human tumors, such as those of sarcoma (Murray et al., 1993a), breast (Murray et al., 1993b), and prostate (Murray et al., 1995). With respect to drug resistance, it is of particular interest that only the invasive part of breast carcinomas has been found to express CYP3A proteins (Murray et al., 1993b). However, these histochemical studies did not distinguish between CYP3A4 and CYP3A5.

In conclusion, we have demonstrated that CYP3A4 metabolizes vinblastine, and that these reactions lead to less toxic compounds. The role of CYP3A4 in the detoxification of vinblastine and vincristine could explain the clinically important idiosyncratic toxicity of these compounds. The detoxification of chemotherapeutic agents by P450-catalyzed metabolism is very unusual because, in most cases, P450s are known to activate a variety of anticancer drugs (Goepart et al., 1994). The only known exception to this appears to be 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) which, as shown only in a cell-free system, is detoxified independently by P450s and by glutathione S-transferases (Weber and Waxman, 1993). In addition, our results strongly suggest that CYP3A4 could be involved in the development of drug resistance during chemotherapy. To establish the significance of these processes in vivo, it will be necessary to examine the P450 expression level in tumor samples derived from patients who have undergone chemotherapy with vinca alkaloids and other chemotherapeutics. The observation that CYP3A4 acts as a selectable marker in the presence of some chemotherapeutic drugs may also provide a mechanism for the induction of CYP3A4 in hepatocytes exposed to taxol (Kostrubsky et al., 1998), which has been shown to be metabolized by this important P450 isoform and by CYP2C8.

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Send reprint requests to: T. Friedberg, Biomedical Research Centre, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK. E-mail: t.h.friedberg@dundee.ac.uk